CLAIMS

What is claimed is:

1. A method for identifying inherited point mutations in a target region of a genome, comprising providing a pool of DNA fragments isolated from a population, and

a) amplifying said target region of each of said fragments in a high fidelity polymerase chain reaction (PCR) under conditions suitable to produce double stranded DNA products which contain a terminal high temperature isomelting domain that is labeled with a detectable label, and where the mutant fraction of each PCR-induced mutation is not greater than about 5×10^{-5};

b) melting and reannealing the product of a) under conditions suitable to form duplexed DNA, thereby producing a mixture of wild type homoduplexes and heteroduplexes which contain point mutations;

c) separating the heteroduplexes from the homoduplexes based upon the differential melting temperatures of said heteroduplexes and said homoduplexes and recovering the heteroduplexes, thereby producing a second pool of DNA that is enriched in target regions containing point mutations;

d) amplifying said second pool in a high fidelity PCR under conditions where only homoduplexed double stranded DNA is produced, thereby producing a mixture of homoduplexed DNA containing wild type target region and homoduplexed DNAs which contain target regions that include point mutations;

e) resolving the homoduplexed DNAs containing target regions which include point mutations based upon the differential melting temperatures

of the DNAs, and recovering the resolved DNAs which contain a target region which includes point mutations; and

f) sequencing the target region of the recovered DNAs to identify point mutations within the target region.

2. The method of Claim 1 wherein said population comprises at least 1000 individuals.

3. The method of Claim 1 wherein said population comprises at least 10,000 individuals.

4. The method of Claim 1 wherein said population comprises between about 10,000 and about 1,000,000 individuals.

5. The method of Claim 1 wherein said population is a population of humans.

6. The method of Claim 5 wherein said human population consists of members of the same demographic group.

7. The method of Claim 6 wherein said human population consists of individuals of European ancestry or a subgroup thereof.

8. The method of Claim 6 wherein said human population consists of individuals of African ancestry or a subgroup thereof.

9. The method of Claim 6 wherein said human population consists of individuals of Asian ancestry or a subgroup thereof.

10. The method of Claim 6 wherein said human population consists of individuals of Indian ancestry or a subgroup thereof.

11. The method of Claim 1 wherein said pool is enriched in fragments containing said target region.

12. The method of Claim 1 wherein said high fidelity PCR is catalyzed by a high fidelity polymerase, and wherein the copy number of each target region in a) is doubled a maximum of about 6 times.

13. The method of Claim 1 wherein the heteroduplexes are separated from the homoduplexes in c), and the homoduplexed DNAs are resolved in d) by constant denaturing gel capillary electrophoresis, constant denaturing gel electrophoresis, denaturing gradient gel electrophoresis or denaturing high performance liquid chromatography.

14. The method of Claim 1 wherein the heteroduplexes are separated from the homoduplexes in c), and the homoduplexed DNAs are resolved in d) by constant denaturing gel capillary electrophoresis.

15. The method of Claim 1 wherein the target region is an isomelting domain consisting of about 80 to about 3,000 base pairs (bp).

16. The method of Claim 1 wherein the target region is about 80 to about 1000 bp.

17. The method of Claim 1 wherein the target region is about 100 to about 500 bp.

18. The method of Claim 1 wherein the target region an exon or portion thereof of a protein encoding gene.

19. The method of Claim 1 wherein the target region spans the junction of an intron and an exon of a protein encoding gene.

20. The method of Claim 1 wherein the target region is a regulatory region of a protein encoding gene.

21. The method of Claim 1 wherein the target region is a intron of a protein encoding gene.

22. The method of Claim 1 wherein said target region is in an gene which encodes RNA.

23. A method for identifying genes which carry a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, determining the frequencies with which each point mutation occurs, and calculating the sum of the frequency of all point mutations identified for each gene or segment;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, determining the frequencies with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment;

c) comparing the sum frequency of point mutations which are found in a selected gene or portion thereof of the young population calculated in a) with the sum frequency of point mutation which are found in the same gene or portion thereof of the aged population calculated in b), wherein a significant decrease in the sum frequency of point mutations in the aged population indicates that said selected gene carries a harmful allele.

24. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 23.

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25. A method for identifying genes which carry a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, and determining the frequencies with which each point mutation occurs;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs; and

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of two or more point mutations in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele.

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26. The method of Claim 25 further comprising:

d) determining the frequency of said two or more point mutations which decrease in the aged population in said selected gene of one or more intermediate age-specific populations;

e) determining the age-specific decline of said two or more point mutations; and

f) comparing the age-specific decline determined in e) with the theoretical age-specific decline of harmful alleles which cause mortal diseases, X(h,t), and determining if the functions are significantly different, wherein a determination that the age-specific decline determined in e) is not significantly different from the theoretical age-specific decline of harmful alleles which cause one or more mortal diseases further indicates that said selected gene carries a harmful allele and has a high probability of being causal of said one or more mortal diseases.

27. The method of Claim 26 further comprising:

g) determining the frequency of said two or more point mutations which decrease in the aged population in said selected gene of one or more proband populations; and

h) comparing the frequencies of said two or more point mutations in said selected gene or portion thereof in the young population with the frequencies of said two or more point mutations in said selected gene or portion thereof in the proband populations; wherein a significant increase in the frequencies of said one or more point mutations in the proband population relative to the young population indicates that said gene caries a harmful allele that plays a causal role in said disease.

28. The method of Claim 26 further comprising:

g) determining the frequency of said two or more point mutations which decrease in the aged population in said selected gene of one or more proband populations consisting of individuals with early onset disease; and

h) comparing the frequencies of said two or more point mutations in said selected gene or portion thereof in the young population with the frequencies of said two or more point mutations in said selected gene or portion thereof in the proband populations; wherein a significant increase in the frequencies of said one or more point mutations in the proband population relative to the young population indicates that said gene carries a harmful allele which is a secondary risk factor which accelerates the appearance of disease.

29. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 25.

30. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 26.

31. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 27.

32. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 28.

33. A method for identifying genes which carry a harmful allele or which are linked to a gene that carries a harmful allele, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, and determining the frequency with which each point mutation occurs;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs;

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant decrease in the frequency of a point mutation in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries a harmful allele or is linked to a gene that carries a harmful allele.

34. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 33.

35. A method of identifying genes which carry a harmful allele that is a secondary risk factor that accelerates the appearance of a disease, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of an early onset proband population, and determining the frequency with which each point mutation occurs;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a late onset proband population, and determining the frequency with which each point mutation occurs;

c) comparing the frequencies of point mutations which are found in a

selected gene or portion thereof in the early onset proband population with the frequencies of the same point mutations in said selected gene or portion thereof of the late onset proband populations; wherein a significant increase in the frequencies of one or more point mutations in the early onset proband population relative to the late onset proband population indicates that said gene carries a harmful allele which is a secondary risk factor which accelerates the appearance of disease.

36. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 35.

37. A method of identifying genes which carries a harmful allele that is a secondary risk factor that accelerates the appearance of a disease, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of an early onset proband population, determining the frequency with which each point mutation occurs, and calculating the sum of the frequency of all point mutations identified for each gene or segment;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a late onset proband population, and determining the frequency with which each point mutation occurs, and calculating the sum of the frequency of all point mutations identified for each gene or segment;

c) comparing the sum frequency of point mutations which are found in a selected gene or portion thereof of the early onset proband population calculated in a) with the sum frequency of point mutation which are found in the same gene or portion thereof of the late onset proband population calculated in b), wherein a significant decrease in the sum frequency of point mutations in the late onset proband population

indicates that said selected gene carries a harmful allele which is a secondary risk factor that accelerates the appearance of a disease.

38. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 37.

39. A method for identifying genes which carry an allele which increases longevity, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, determining the frequencies with which each point mutation occurs, and calculating the sum of the frequency of all point mutations identified for each gene or segment;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, determining the frequencies with which each point mutation occurs, and calculating the sum of the frequencies of all point mutations identified for each gene or segment;

c) comparing the sum frequency of point mutations which are found in a selected gene or portion thereof of the young population calculated in a) with the sum frequency of point mutation which are found in the same gene or portion thereof of the aged population calculated in b), wherein a significant increase in the sum frequency of point mutations in the aged population indicates that said selected gene carries an allele which increases longevity.

40. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 39.

41. A method for identifying genes which carry an allele which increases longevity, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, and determining the frequencies with which each point mutation occurs;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs; and

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant increase in the frequency of two or more point mutations in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries an allele which increases longevity.

42. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 41.

43. A method for identifying genes which carry an allele which increases longevity or which are linked to a gene that increases longevity, comprising:

a) identifying the inherited point mutations which are found in the genes or portions thereof of a population of young individuals, and determining the frequency with which each point mutation occurs;

b) identifying the inherited point mutations which are found in the genes or portions thereof of a population of aged individuals, and determining the frequency with which each point mutation occurs;

c) comparing the frequency of each point mutation identified in a selected gene or portion thereof of the young population determined in a) with the frequency of the same point mutations identified in said selected gene of the aged population determined in b), wherein a significant increase in the frequency of a point mutation in said selected gene of the aged population relative to said selected gene of the young population indicates that said selected gene carries an allele which increases longevity or is linked to a gene that increases longevity.

44. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 43.

45. A method for identifying genes which affect the incidence of a disease, comprising:

a) identifying the inherited point mutations which are found in genes or portions thereof of a population of young individuals not afflicted with said disease, determining the frequencies with which each point mutation occurs, and summing the frequency of all point mutations identified in each gene or segment thereof;

b) identifying the inherited point mutations which are found in genes or portions thereof of a proband population having said disease, determining the frequencies with which each point mutation occurs, and summing the frequency of all point mutations identified in each gene or segment thereof;

c) comparing the sum frequency of point mutation in a selected gene or portion thereof in the young population with the sum frequency of point mutations in said selected gene or portion thereof in the proband population; wherein a significant increase in the sum frequency of point mutations in the proband population indicates that said gene plays a causal role in said disease.

46. The method of Claim 29 wherein said disease is a mortal disease.

47. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 45.

48. A method for identifying a gene which carries deleterious alleles, comprising

a) identifying the inherited point mutations occurring in the exon(s) and splice sites of said gene of a population of young individuals;

b) identifying the subset of point mutations in a) that are obligatory knockout point mutations, and determining the frequencies with which each obligatory knockout point mutation occurs; and

c) summing the frequency of all obligatory knockout point mutations identified in the gene; wherein a sum frequency of less than about 2% indicates that said gene carries a deleterious allele.

49. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 48.

50. A method for identifying a gene which carries deleterious alleles, comprising

a) identifying the inherited point mutations occurring in the exon(s) and splice sites of said gene of a population of young individuals;

b) identifying the subset of point mutations in a) that are obligatory knockout point mutations, and determining the frequencies with which each obligatory knockout point mutation occurs;

c) identifying the subset of point mutations in a) that are presumptive knockout point mutations, and determining the frequencies with which each presumptive knockout point mutation occurs; and

d) summing the frequency of all of said obligatory knockout point mutations and presumptive knockout point mutations identified in the gene; wherein a sum frequency of less than about 2% indicates that said gene carries a deleterious allele.

51. The method of Claim 32 wherein a sum of about 0.02% to about 2% indicates that said gene carries a recessive deleterious allele.

52. The method of Claim 32 wherein a sum of less than about 0.02% indicates that said gene carries a dominant deleterious allele.

53. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 50.

54. A method for isolating and identifying a target region of a genome which contains inherited point mutations, comprising providing a pool of DNA fragments isolated from a population, and

a) amplifying said target region of each of said fragments in a high fidelity polymerase chain reaction (PCR) under conditions suitable to produce double stranded DNA products which contain a terminal high temperature isomelting domain that is labeled with a detectable label, and where the mutant fraction of each PCR-induced mutation is not greater than about 5×10^{-5};

b) melting and reannealing the product of a) under conditions suitable to form duplexed DNA, thereby producing a mixture of wild type homoduplexes and heteroduplexes which contain point mutations;

c) separating the heteroduplexes from the homoduplexes based upon the differential melting temperatures of said heteroduplexes and said homoduplexes and recovering the heteroduplexes, thereby producing a second pool of DNA that is enriched in target regions containing point mutations;

d) amplifying said second pool in a high fidelity PCR under conditions where only homoduplexed double stranded DNA is produced, thereby producing a mixture of homoduplexed DNA containing wild type target region and homoduplexed DNAs which contain target regions that include point mutations;

e) resolving the homoduplexed DNAs containing target regions which include point mutations based upon the differential melting temperatures of the DNAs, and recovering the resolved DNAs which contain a target region which includes point mutations; and

f) sequencing the target region of a recovered DNA which contain a target region which include point mutations.

55. An isolated target region of a genome which contains an inherited point mutations isolated by the method of Claim 54.

56. An isolated nucleic acid which is complimentary to a strand of a gene or allele thereof identified by the method of Claim 54.

57. An array of isolated nucleic acids, immobilized on a solid support, said array having at least about 100 different isolated nucleic acids which occupy separate known sites in said array, wherein each of said different isolated nucleic acids hybridizes to a target region which contains an inherited point mutation of Claim 54.

58. An array of isolated nucleic acids, immobilized on a solid support, said array having at least about 100 different isolated nucleic acids which occupy separate known sites in said array, wherein said array comprises all known deleterious, harmful and beneficial point mutations for all human populations.

59. A method of identifying the inherited point mutations in any target region of a genome of a population, wherein said point mutations

a) interfere with reproduction;

b) cause or accelerate the appearance of a mortal disease; or

c) prevent or delay the appearance of a mortal disease; wherein

the set of all inherited point mutations occurring at a frequency at or above 5 x10-5 is first identified separately in members of the same population who comprise subpopulations selected from the group consisting of young, aged, intermediate age, afflicted with disease, afflicted with a disease of early age onset and afflicted with a disease of late age onset, by noting the frequencies of each inherited point mutation within and between the subpopulations, thereby determining which inherited point mutations are deleterious, harmful or beneficial.

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